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Authors

Pinkerton, KE
Green, FH
Saiki, C
et al.

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Distribution of Particulate Matter and Tissue Remodeling in the Human Lung

Kent E. Pinkerton,[1] Francis H.Y. Green,[2] Cathy Saiki,[1] Val Vallyathan,[3] Charles G. Plopper,[1] Venu Gopal,[4] Daniel Hung,[1] Emily B. Bahne,[1] Susan S. Lin,[1] Margaret G. Ménache,[5] and Marc B. Schenker[1]

[1]Department of Anatomy, Physiology, and Cell Biology, School of Veterinary Medicine and Department of Epidemiology and Preventive Medicine, School of Medicine, University of California, Davis, California, USA; [2]Department of Pathology and Laboratory Medicine, Faculty of Medicine, University of Calgary, Calgary, Alberta, Canada; [3]National Institute for Occupational Safety and Health, Morgantown, West Virginia, USA; [4]Fresno Medical Coroner's Office, Fresno, California, USA; [5]Department of Pediatrics, School of Medicine, University of New Mexico, Albuquerque, New Mexico, USA

We examined the relationship between intrapulmonary particle distribution of carbonaceous and mineral dusts and remodeling of the airways along anatomically distinct airway paths in the lungs of Hispanic males from the central valley of California. Lung autopsy specimens from the Fresno County Coroner's Office were prepared by intratracheal instillation of 2% glutaraldehyde at 30 cm H_2O pressure. Two distinct airway paths into the apico-posterior and apico-anterior portions of the left upper lung lobe were followed. Tissue samples for histologic analysis were generally taken from the intrapulmonary second, fourth, sixth, and ninth airway generations. Parenchymal tissues beyond the 12th airway generation of each airway path were also analyzed. There was little evidence of visible particle accumulation in the larger conducting airways (generations 2–6), except in bronchial-associated lymphoid tissues and within peribronchial connective tissue. In contrast, terminal and respiratory bronchioles arising from each pathway revealed varying degrees of wall thickening and remodeling. Walls with marked thickening contained moderate to heavy amounts of carbonaceous and mineral dusts. Wall thickening was associated with increases in collagen and interstitial inflammatory cells, including dust-laden macrophages. These changes were significantly greater in first-generation respiratory bronchioles compared to second- and third-generation respiratory bronchioles. These findings suggest that accumulation of carbonaceous and mineral dust in the lungs is significantly affected by lung anatomy with the greatest retention in centers of lung acini. Furthermore, there is significant remodeling of this transitional zone in humans exposed to ambient particulate matter. *Key words*: **asthma, California, fibrosis, lung pathology, $PM_{2.5}$, PM_{10}, particulate matter, pigmentation.** *Environ Health Perspect* **108:1063–1069 (2000). [Online 23 October 2000]**
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Exposure to airborne particles is a common event. Most particles that are respired are readily removed by mucociliary clearance aided by macrophage phagocytosis (*1*). A smaller fraction is retained within lung tissues or redistributed to regional lymph nodes (*2*). The deposition and clearance of particles within the respiratory system occurs in an inhomogeneous manner. The fate of particles is not well established, and there is little information on the distribution and retention of particles under conditions of ambient exposure. One purpose of this study was to design and implement an approach that would allow the assessment of particle retention as well as histologic analysis of response at different levels of the lung. This paper describes the methodology for preparing tissue and sampling airways and gas-exchange regions along precisely defined airway paths.

The environment of the central valley of California places individuals at increased risk of exposure to particles. This region encompasses a rich farming area as well as extensive urban development. The interface between rural and urban environments creates a unique setting for exposure to both natural and anthropogenic sources of airborne particulate matter. The predominantly dry farming techniques of the central valley result in high levels of airborne dust from operations such as field preparation and harvesting of row crops and tree fruits (*3,4*). Combustion exhaust particles may also arise from equipment used in agricultural operations. Urban sprawl also generates an abundance of combustion and secondary photochemical gases and airborne particles.

We have had the opportunity over the past 5 years to examine lung specimens from deceased young Hispanic males through the Fresno County Coroner's Office. Approximately 50% of these individuals are known to have been farmworkers; the remaining individuals were employed in nonfarming operations. These individuals were healthy and considered to be free of pulmonary disease, having died from nonrespiratory causes.

Our objective was to examine the relationship between retained carbonaceous and mineral dust in the lungs and the remodeling of the small airways along the same airway paths of each individual. In this paper, we report evidence that both carbonaceous and mineral dust are primarily distributed to the terminal and respiratory bronchioles and that there is anatomical remodeling within these same sites.

Methods

Population. Left lungs from 42 autopsies of Hispanic males were collected at the Fresno County (California) Coroner's Office from June 1994 to June 1995. Demographic information, limited smoking, and occupational histories were obtained from the medical examiner and the coroner's files. The study subjects ranged in age from 18 to 73 years and had died suddenly or unexpectedly. An autopsy was performed at the coroner's office to determine the cause and manner of death as dictated by state statute. Complete autopsies were performed within a period of 12–24 hr after death. The project was reviewed and approved by the Human Subjects Review Committee of the University of California, Davis (UC-Davis).

Preparation. The left lung of each deceased individual was cannulated through the left mainstem bronchus and inflation-fixed with 2% glutaraldehyde at a hydrostatic pressure of 30 cm of water for a period of 2 hr from a constant-pressure gravity apparatus. The lungs were cut in the sagittal plane to include the mainstem bronchus, hilar structures, and the medial aspect of both the upper and lower lobes of the left lung. Each specimen was subsequently stored in fixative and shipped to UC-Davis. Upon arrival, we photographed each lung from the cut sagittal surface as well as a medial view (Figure 1A,B).

Address correspondence to K.E. Pinkerton, Department of Anatomy, Physiology, and Cell Biology, University of California, 1321 Haring Hall, Davis, CA 95616-5270 USA. Telephone: (530) 752-8334. Fax: (530) 752-7690. E-mail: kepinkerton@ucdavis.edu

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We also examined each lung and documented selected gross features on a standard form. These included pleural pigmentation and thickening, the appearance and size of the tracheobronchial lymph nodes, hemorrhage, and fibrosis. Emphysema was graded on gross photographs of the whole lung by the method of Thurlbeck (*5*). The airways were examined for mucous plugs or aspirated material within the lumen.

Airway microdissection. Beginning at the left mainstem bronchus, the airways were microdissected using razor blades, scissors, and a dissecting microscope along two pathways leading to the apico-posterior and apico-anterior portions of the left upper lobe (Figure 1C,D). Details of the dissection approach and the airway recordings and numbering system have been previously described (*6*). We performed the microdissections in an identical, systematic way for all 42 cases. The dimensions and orientation (length, diameter, and branching angle) were measured in all generations along the two paths (Figure 2). The microdissection created complementary halves of each airway (Figure 1C). These were examined with a dissecting microscope for visible pigment distribution, and tissue samples for histologic analysis were generally taken from the second, fourth, sixth, and ninth airway generations. We also sampled parenchymal tissues and associated terminal and respiratory bronchioles were also sampled beyond the 12th airway generation of each airway path that was microdissected.

Pathologic evaluation. Samples of airways of varying size and airway generation, together with samples of pulmonary parenchyma, were taken from representative areas of the upper and lower lobes for the overall evaluation of pathologic changes. Each tissue block was embedded in paraffin (Fischer Scientific, Fair Lawn, NJ), and 5-µm thick sections were cut using a rotary microtome and stained with hematoxylin and eosin. We also used sirius red and elastic trichrome stains to confirm the presence of collagen and smooth muscle within tissue sections.

We applied standard diagnostic criteria for the recognition of pneumoconiotic lesions (*7–10*). The following histologic features were evaluated using a semiquantitative scale: macules, defined as collections of dust-laden macrophages in a size range of 0.1–0.6 mm within the walls of respiratory bronchioles and adjacent alveoli; nodules, defined as fibrotic lesions up to 1 cm in size with round, irregular, or serpiginous borders and containing dust-laden macrophages; and interstitial fibrosis, defined as diffuse or irregular fibrosis of alveolar septa and/or alveolar ducts.

We evaluated tracheobronchial lymph nodes for the presence of fibrosis and dust. Chronic bronchitis and asthma were evaluated using standard pathologic criteria (*7*). Small airways disease, subdivided into mineral dust-associated small airways disease (*11*) and smoking-related small airways disease (*12,13*), was identified, and exposure to cigarette smoke in the recent past was assessed on the basis of accumulation of characteristic smokers' macrophages within the respiratory bronchioles and adjacent alveoli (*14,15*).

Structural remodeling of small airways. The microdissection technique and preliminary analyses of tissue sections indicated that small airways were the primary retention site for particles. For the semiquantitative

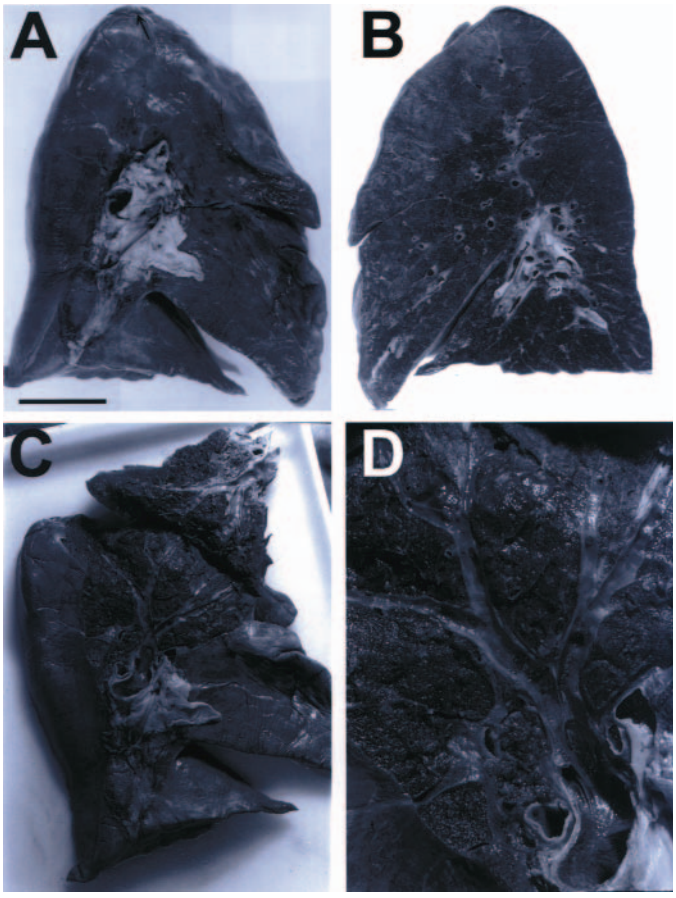

Figure 1. Medial surface of the left human lung (*A*) and the cut, sagittal surface of the same lung (*B*). Several small apical blebs (arrow) were noted in this lung. Anatomically distinct airway paths beginning at the left mainstem bronchus are followed to the apico-posterior and apico-anterior regions of the left upper lobe (*C*). A magnified view of the microdissected airways (*D*). Scale bar in (*A*) = 5 cm.

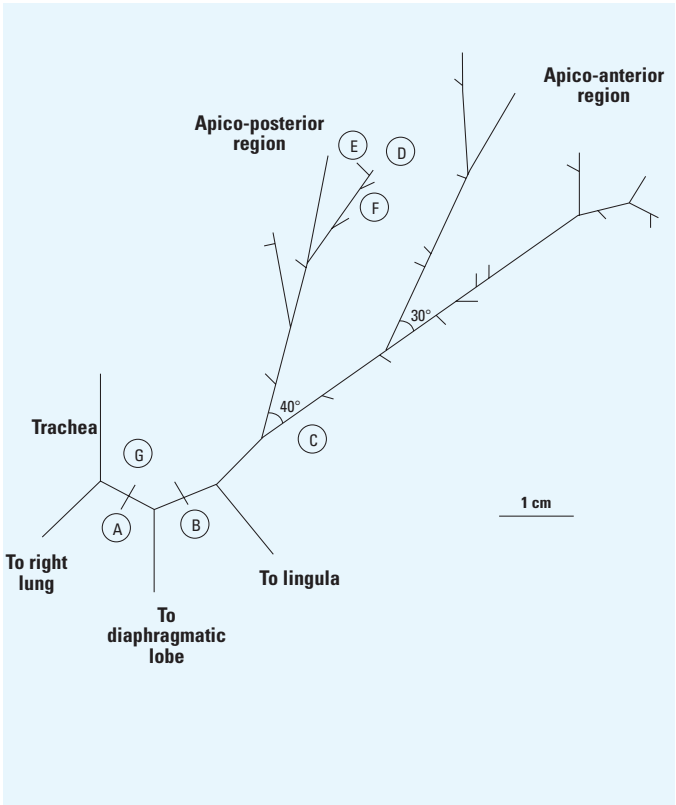

Figure 2. Stick diagram of the airway branching pattern from the left upper lobe of a 45-year-old farm laborer (employed for 15 years) who had lived for 17 years in Fresno County. He was a nonsmoker. Beginning at the left mainstem bronchus, airway paths leading to the apico-posterior and apico-anterior regions of the lungs were followed by microdissection. Letters in circles indicate sites taken for embedment and histologic examination. A, second-generation airway (left mainstem bronchus); B, third-generation airway; C, fifth-generation airway; D,E,F, first-generation respiratory bronchioles beyond the tenth-generation airway; G, hilar lymph node.

evaluation of small airways, six samples per case were taken from the left upper lobe to include the ventilatory zones of the airway paths that had been microdissected. Serial sections were stained with hematoxylin and eosin or elastic trichrome for connective tissue (collagen and elastic fibers), and smooth muscle. The following airways were evaluated: membranous (terminal) bronchioles, first-generation respiratory bronchioles, second-generation respiratory bronchioles, and third-generation respiratory bronchioles. In each serial section, we graded all membranous bronchioles cut in a plane perpendicular to the longitudinal profile of the airway. All orientations of respiratory bronchioles were included; however, the generation of each airway level was clearly identified based on position from the terminal bronchiole and/or first-generation respiratory bronchiole. The orientation (longitudinal, oblique, or cross-section) was recorded. We evaluated the airways for fibrosis, muscle hypertrophy, inflammation, visible and polarizable pigment, and intraluminal macrophages. Because the degree of epithelial loss from autolysis was variable, we did not attempt semiquantitative analysis of this feature. Each of the features was graded from 0 to 3, where 0 represented no evidence of that feature, and 1–3 represented increasing grades of severity. The categories of severity were based on preliminary review of the material to assess the range of change present. Typical examples of each grade were photographed for periodic review. Variability of the readings and interobserver reproducibility were established by having three readers (F.H.Y.G., K.E.P., and V.V.) each independently evaluate the same 30 histologic slides on two different occasions.

Statistical analysis. We analyzed 42 cases for this study. To compare the degree (score) of histologic change within specific levels of the lungs, particularly for the first-, second-, and third-generation respiratory bronchioles, we used the Friedman test. To increase the power of this analysis, only cases with three or more complete sets of respiratory bronchioles consisting of contiguous first-, second-, and third-generation bronchioles were used. Of the 42 cases, 28 cases met this criteria. We used linear regression analysis to compare scores of histologic features with scores of the abundance of particles (i.e., carbonaceous substances and crystalline dust). Spearman correlation coefficients were also used to examine the relationship between particle burden and histologic change within each region of the lungs. A p-value < 0.05 was considered to be statistically significant. We evaluated interobserver variables for the grading of respiratory bronchioles with the kappa statistic (*16*).

Results

The demographic profile of the study population is shown in Table 1. All cases were Hispanic males; 34% had lived in Fresno County up to 10 years, 37% for 11–20 years, 20% for 21–30 years, and 9% had lived in the region for more than 30 years. In general, subjects were young, with a median age of 33 years (range 18–73 years). The majority of subjects worked in farming or had other blue-collar occupations. Approximately half of the subjects were current smokers at the time of death (Table 1). Cause of death by the *International Classification of Diseases, Revision 9, Clinical Modification* (ICD-9CM) is shown in Table 2. As these were coroner's cases, the majority of deaths were sudden or unexplained. Deaths due to vehicular accidents, cardiovascular disease, homicide, and suicide predominated.

Table 3 shows the major pathologic abnormalities in the lungs of this population. On gross examination, with the exception of hemorrhage, almost no lungs showed visible evidence of disease. Only one case, a 44-year-old smoker, showed grossly visible emphysema in the form of several small apical subpleural blebs (Figure 1A,B). There was variable visible pigmentation, mostly along lymphatics in the subpleural interlobular septa, but also in some cases in the centriacinar zones and along the bronchopulmonary airways. Black pigment was also seen within tracheobronchial lymph nodes, which were variably enlarged.

A majority of normal-appearing lungs on gross examination displayed subtle but recognizable abnormalities at the microscopic level (Table 3). Approximately half of the subjects showed histologic evidence of smoking-related lung injury; i.e. chronic bronchitis and small airways disease. Smoking-related small airway disease and mineral dust-associated small airways diseases were seen in 45% and 26% of cases, respectively. Pneumoconiosis was observed (macules) in 4 subjects (10%), lymph node fibrosis associated with mineral dust in 16 (38%), and asthma in 14 (33%) of the 42 subjects (Table 3).

A typical microdissection pathway is shown schematically in Figure 2. The left mainstem bronchus (second generation airway) as well as the third, fifth, ninth, and twelfth airway generations were sampled. The number of airway generations to parenchymal regions sampled ranged from 8 to 15. Parenchymal tissues arising from the apico-posterior and apico-anterior regions were examined for membranous and respiratory bronchioles. Microscopic evaluation of the microdissected airways revealed in many instances that the epithelial lining layer of the airway had sloughed. In spite of this loss, the interstitial wall of the airway as well as submucosal glands were preserved.

Carbonaceous pigment was localized in and around lymphatic vessels in the adventitial portions of the airways and within the attendant lymph nodes. However, only rarely were carbonaceous materials or birefringent particles identified immediately beneath the epithelial lining of the airways or within the bronchial wall, and in these circumstances the amounts were small (Figure 3A–C). In contrast, at the level of the membranous and respiratory bronchioles, the presence of carbonaceous materials as well as birefrigent particles was noted in the majority of cases (Figure 3D–F). These particles were particularly abundant and heavy in the adventitial wall of membranous bronchioles (Figure 4) and within the walls of first-generation respiratory bronchioles. The pigment and birefringent particles in the walls of the respiratory bronchiole were primarily seen around the

Table 1. Demographic characteristics of population.

Characteristic	Number or frequency
Population	42
Smokers	19 (45%)
Nonsmokers	23 (55%)
Median age (25th–75th quartiles)	33 (24–53)

Table 2. Cause of death.

Cause of death	ICD-9CM code range	Frequency
Heart and cardiovascular disease/natural causes	E420–429	8 (19%)
All vehicle accidents	E800–E848	16 (38%)
Accidental poisonings by psychotropic agents	E854	3 (7%)
Accidental drowning and submersion	E910	1 (2%)
Agricultural machine accident	E919	1 (2%)
Suicide and self-inflicted injury	E950–E959	4 (10%)
Homicide and injury inflicted by others	E960–E969	6 (15%)
Unknown cause	—	3 (7%)

Table 3. Lung pathology in 42 residents of Fresno County, California.

Specific feature	Prevalence
Mineral dust small airways disease	11 (26%)
Smoking-related small airways disease	19 (45%)
Pneumoconiosis (macules and/or nodules)	4 (10%)
Interstitial fibrosis	4 (10%)
Lymph node fibrosis	16 (38%)
Chronic bronchitis	21 (50%)
Asthma	14 (33%)
Emphysema	1 (2%)

attendant pulmonary artery, but also extended into the peribronchiolar tissues and adjacent alveolar septal walls. In first-generation respiratory bronchioles, dust retention was greatest in the nonalveolarized portion of the wall (Figure 3D–F). Heavy particle distribution was also observed within bronchial lymph nodes (Figure 3G). Here the dust was located within reticular cells lining the sinuses. Fibrosis, associated with visible and birefringent particles, was observed in 38% of the case population (Figure 4).

The distribution of particles and histologic features of the membranous and respiratory bronchioles were further analyzed in respiratory bronchioles, in longitudinal profile, which could be identified as either first, second, or third generation (Figure 5). Of the 42 original cases, 28 contained a minimum of 3 sets of respiratory bronchioles, each consisting of first-, second-, and third-generation segments. Based on this sample power, an analysis was done to score for changes within each of these three generations of respiratory bronchioles from each case for the following histologic features: smooth muscle hypertrophy, fibrosis, alveolar macrophage frequency, degree of inflammatory change within the interstitial wall, amount of carbonaceous pigment within each respiratory bronchiole generation, and the amount of birefringent dust particles within each of these generations. Membranous bronchioles within the same tissue sections were evaluated for the same features. For all histologic analysis and scoring, there was reasonable agreement between the three observers, with interobserver variability ranging from 64.0% to 73.0%, for complete agreement with kappa of 0.42–0.57.

The results of the histologic and particle analysis of respiratory bronchioles by order of generation are given in Figure 6. There was a highly significant difference ($p < 0.001$) in the degree of histologic changes for all features examined in first-generation respiratory bronchioles, compared to second-generation respiratory bronchioles, as well as changes in second-generation compared to third-generation respiratory bronchioles ($p < 0.001$). The amount of pigment in terminal bronchioles was significantly correlated with the amount of pigment in respiratory bronchioles, particularly for mineral dusts (linear regression analysis, $R^2 = 0.66$, $p < 0.05$). Multiple variable linear regression analysis of the relationship between pigment and airway fibrosis revealed that pigment was powerful predictor of the degree of fibrosis in the terminal bronchioles and all three generations of respiratory bronchioles. Scatterplots of fibrosis versus pigmentation scores for first-, second-, and third-generation respiratory bronchioles are shown in Figure 7.

Discussion

Situated in the heart of the San Joaquin Valley, Fresno has some of the highest inhalable particle concentrations (particulate matter ≤ 10 µm; PM_{10}) in the United States, often exceeding the National Ambient Air Quality Standard of 150 µg/m^3 averaged over 24 hr. The physicochemical characteristics and seasonal variability of PM at Fresno have been described in detail by Chow et al. (*17,18*). During the winter months, the highest PM_{10} concentrations have a dominant

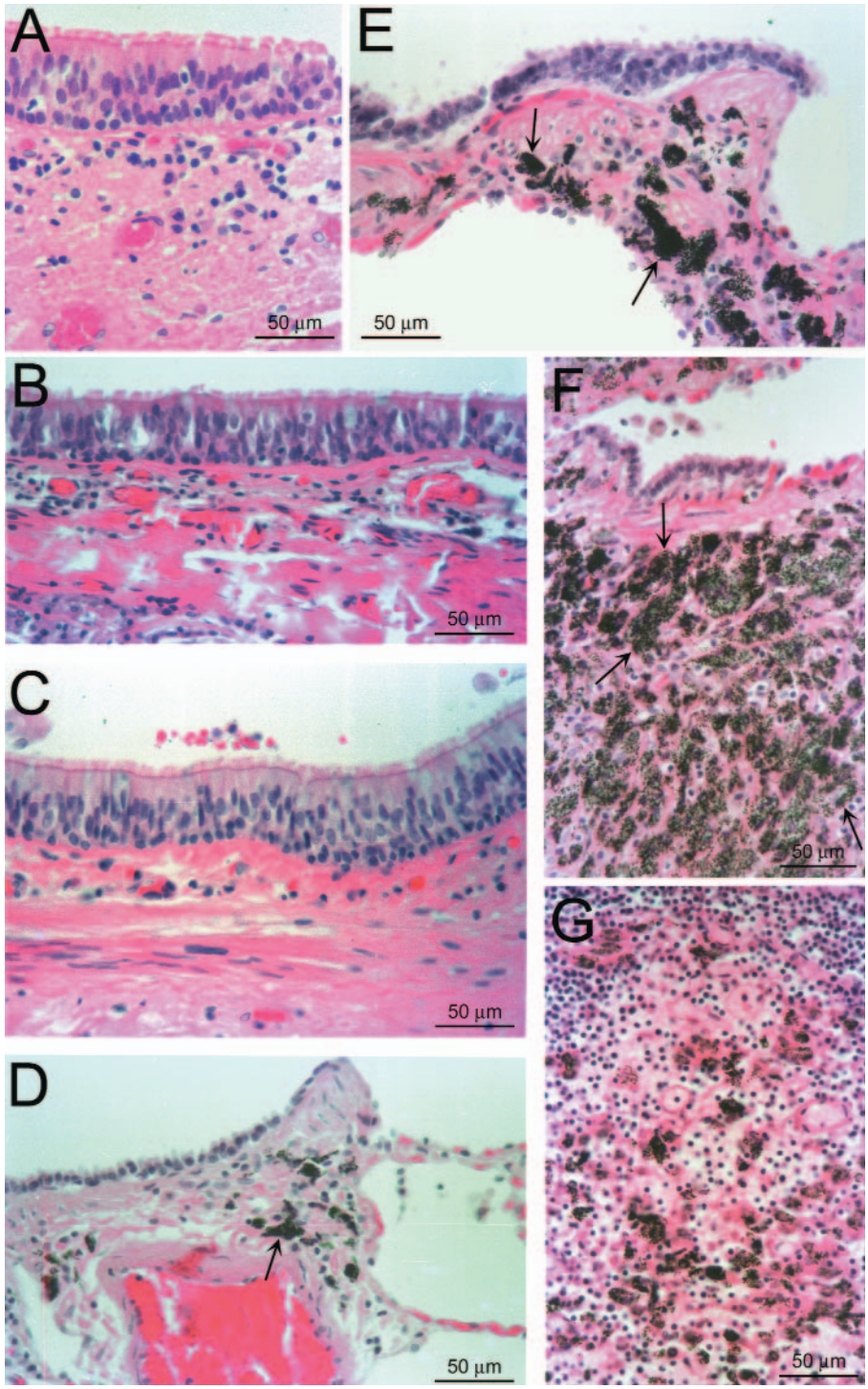

Figure 3. Light micrographs of the second- (*A*), third- (*B*), and fifth- (*C*) generation airways, first-generation respiratory bronchioles (*D,E,F*), and bronchial lymph node (*G*) from the same lung. The location of each anatomical site is illustrated in Figure 2. Particles were not evident within the interstitial wall of the second-, third-, or fifth-generation airways. However, significant amounts of black pigment were noted within the first-generation respiratory bronchioles (arrows; *D,E,F*). Scoring for black pigment was 1 in (*D*), 2 in (*E*), and 3 in (*F*) (see text).

$PM_{2.5}$ fraction (i.e., fraction of particles < 2.5 μm). These particles consist mostly of carbonaceous constituents (especially particles < 0.3 μm) as well as ammonium nitrate and sulfate (0.3–2.5 μm). Elevated PM_{10} concentrations during the summer and early fall occur due to windblown dust excursions, which have been most often found in the southern San Joaquin Valley and in the high desert regions. These situations are dominated by fugitive dusts mostly associated with coarse (i.e., 2.5–10 μm) particles.

Due to these conditions of ambient particulate matter in the Fresno area, the examination of human lung autopsies from this region for the distribution of carbonaceous materials and mineral dusts in the lungs is highly relevant. The cases examined were all Hispanic males who had lived an average of 16 years in Fresno County. Approximately one-half of these subjects were farmworkers, while the others were in blue-collar occupations. In all instances, these individuals did not die of respiratory causes and most were in apparent good health before death. Although occupational (agricultural) exposures to dusts could have been significant in some cases, this study should be considered highly appropriate to better understand the possible biological effects of ambient particulate matter in the lungs and the distribution of particles in the respiratory tract of otherwise healthy individuals. In no instance did an individual die due to respiratory disease.

From 14 June 1994 through 9 June 1995, the time during which autopsy material was collected for this study, California and federal 24-hr and annual standards for PM_{10} were regularly exceeded in Fresno County at both urban and nonurban sites (*19*). During this time, the PM_{10} daily (arithmetic) average concentration in Fresno was 43.5 μg/m^3, and the maximum 24-hr PM_{10} concentration was 122 μg/m^3. The corresponding levels of $PM_{2.5}$ were 22 and 65 μg/m^3. Oxidant gases were also measured during this same period. One-hour measurements of nitrogen oxides (NO_x) averaged 0.109 ppm, with maximal levels reaching 0.7 ppm. The average 1-hr concentration of ozone during this time was 0.06 ppm, while the maximum 1-hr ozone concentration was 0.17 ppm. Sulfur oxide levels averaged 0.0054 ppm with a maximal concentration of 0.017 ppm (*19*). Exposures from occupational tasks are not known for individual cases, but it is unlikely that this would have affected particle distribution within the lungs examined in this study.

In this study, we used a systematic approach to determine the distribution of ambient particles in the human lung and their potential role in tissue remodeling. This approach involved dissection of defined airway paths and parenchymal sampling in adjacent regions. As a result of this sampling procedure, the importance of terminal and respiratory bronchioles as sites for particle retention and the association of particle retention with subtle but quantifiable changes in tissue remodeling have been clearly established.

Remodeling of lung tissues in a number of cases was reminiscent of changes observed in nonhuman primates after exposure to

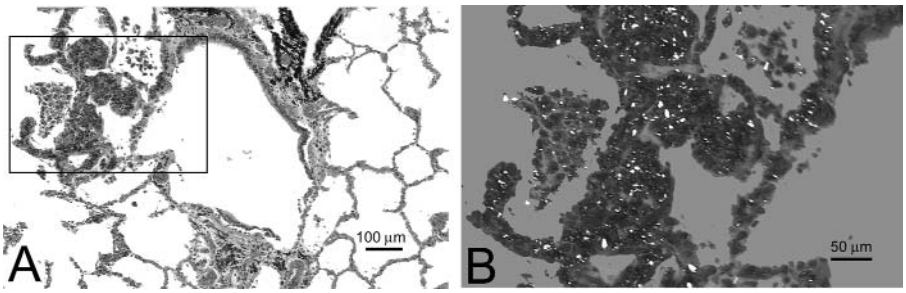

Figure 4. Bright field (*A*) and polarized field (*B*) images of a first-generation respiratory bronchiole from a 32-year-old farm laborer who had hypertrophic heart disease. Alveolar outpocketings are present in the walls of this airway. Within adjacent alveoli are accumulations of intraluminal macrophages containing numerous polarized particles. Some of these particles are also present in the walls of the respiratory bronchiole and neighboring alveoli.

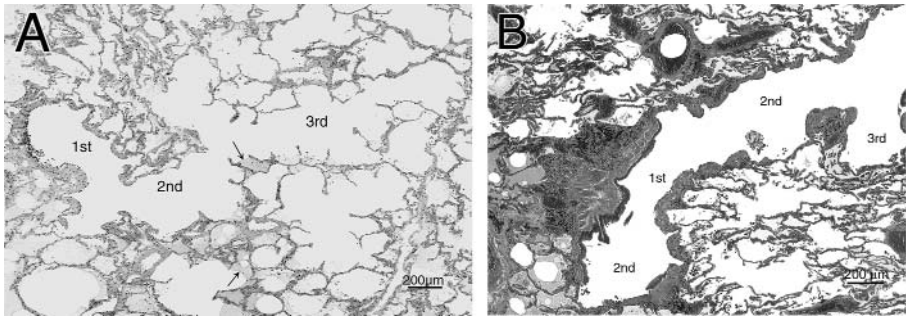

Figure 5. (*A*) Contiguous first-, second-, and third-generation respiratory bronchioles in longitudinal profile from the lung of a 69-year-old male who had spent 37 years as a farmer and had lived 50 years in Fresno County, California. Postmortem changes and alveolar filling with edema fluid are present (arrows). These postmortem changes did not affect grading of histologic features within airway walls, but loss of epithelium due to autolysis in this as well as other cases precluded analysis of this feature. (*B*) Three generations of respiratory bronchioles from a 30-year-old male from Fresno County, showing severe grades of pathologic change in the respiratory bronchioles. Specifically, there are increases in collagen, smooth muscle, and visible pigment. Also note that the changes are maximal in the first-generation respiratory bronchiole, with a progressive decrease in these tissue responses in more distal generations.

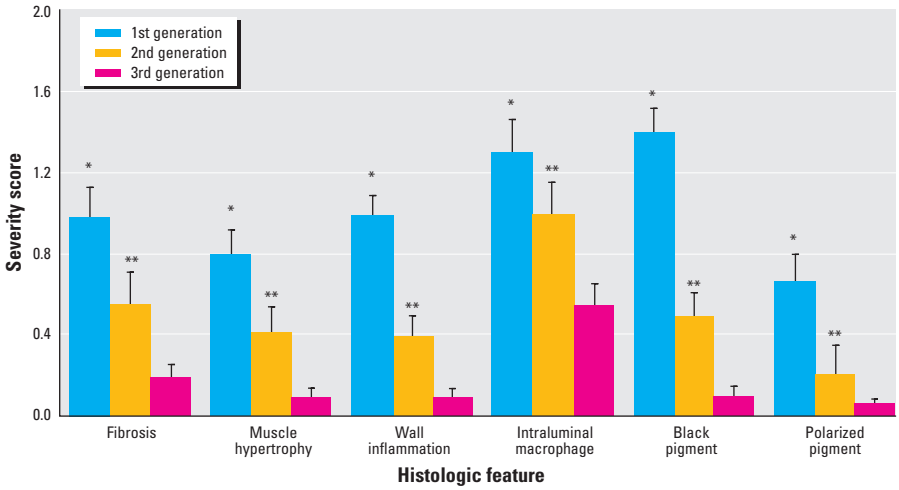

Figure 6. Histogram showing the relationship between the severity scores for specific histologic features by respiratory bronchiolar generation. The data indicate an autocorrelation between histologic features (see text for more details).

*For all features, severity scores were significantly greater ($p < 0.001$) in first-generation respiratory bronchioles compared to the second and third generations. **All scores for second-generation respiratory bronchioles were significantly greater ($p < 0.001$) than scores for the third-generation respiratory bronchioles.

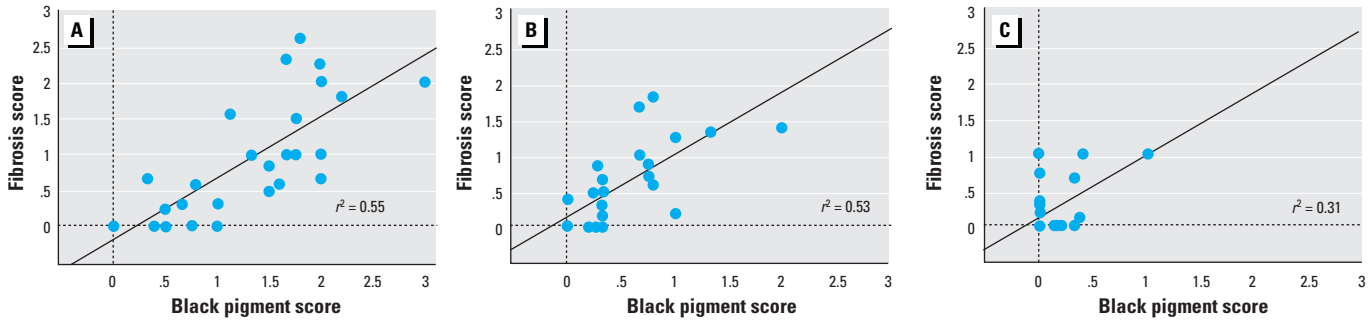

Figure 7. Scatterplot of regression analysis for fibrosis score to black pigment score of first- (*A*), second- (*B*), and third- (*C*) generation respiratory bronchioles. A positive correlation is seen for all three airways, but it is greatest for first- and second-generation respiratory bronchioles.

ambient concentrations of ozone (*20*), the primary oxidant gas of photochemical air pollution. Predominant pathological effects were also confined to the epithelial and interstitial tissue compartments of the respiratory bronchioles, forming the transitional zone between the conducting airways and gas-exchange regions of the lungs. The importance of this site as a target for particle-induced injury is well established in occupational settings (*21*). The centriacinar region is the primary site of injury in coal worker's pneumoconiosis (*8*), asbestosis (*9*), and silica- and silicate-induced injury (*10*). Silicate pneumoconiosis has also been found in limited numbers of farmworkers from the central valley of California (*22*). It has also been known for many years that cigarette smoking induces injury in this region in young smokers and that respiratory bronchiolitis precedes the functionally more important disease, centriacinar emphysema, often by several decades (*14,15*). However, it is not generally recognized that subtle lesions can occur in the centriacinar zone of the lung in individuals exposed to ambient particles. Our results show a continuum of changes in the respiratory bronchioles, being most severe in individuals who smoke, but also present to a lesser degree in the population in general. These lesions have physiologic effects that can only be detected by nonroutine tests for small airway function (*15*). They may progress to clinically significant disease status (*15*).

Analytical electron microscopic studies by Churg and colleagues (*23*) have revealed that the concentration of particles in respiratory bronchioles can be 25–100 times greater than the concentration of particles in the mainstem bronchus (*23*). This finding is in keeping with our own observations. Churg et al. (*23*) also noted high particle concentrations at the bifurcations of airway generations 4 and 5. We did not observe large accumulations of visible dust at these sites, but this may be due to the fact that our technique identified only particles in tissue sections rather than particle number recovered by complete tissue digestion. Churg and Brauer (*24*) have also studied the size of particles in the pulmonary parenchyma (which includes the respiratory bronchiolar region) and have shown that the geometric mean particle diameter was 0.38 µm. Conversion of the projected diameters to aerodynamic diameters revealed that 96% of the particles had aerodynamic diameters < 2.5 µm. These data, taken in conjunction with our own observations, indicate that it is the finer particles (< 2.5 µm diameter) that are responsible for tissue remodeling.

Our data also show marked differences between particle retention and remodeling in the first-, second-, and third-generation respiratory bronchioles. The major site of impact and injury appeared to be the terminal bronchiole and adjacent first-generation respiratory bronchioles, with progressive decrease in both retention of particles and injury in the second- and third-generation respiratory bronchioles. This region of the lung is anatomically distinct, as the respiratory bronchioles have some of the same properties of conducting airways as well as serving a gas-exchange function (*25*). Furthermore, this region is the point of entry and exit to the ventilatory unit; gases and airborne particles entering and leaving the acinus have to pass through this constricted zone. Particles deposited within the acinus are also cleared through this portal within macrophages. Thus, the region is unusually vulnerable to the effects of gases and particles impacting the airway wall and to the influence of macrophages and neutrophils containing ingested particles.

Models of particle (aerosol) deposition have demonstrated the importance of respiratory bronchiole and alveolar duct structures in particle deposition (*26–29*). It is also a region with highly specialized cells, including alveolar epithelial cells (type I and II), ciliated cells, nonciliated bronchiolar epithelial (Clara) cells, neuroendocrine cells, and others which may be particularly vulnerable to damage associated with particle retention. In this region, chaotic mixing may be the important mechanism of particle deposition and dispersion. The anatomical makeup of this region suggests that there is potential for clearance overload with enhanced deposition and subsequent retention of particles in this zone. This appears to be the mechanism for development of the macular lesions of coal worker's pneumoconiosis (*7*).

The relative absence of retained particulate matter in the larger conducting airways probably reflects more rapid clearance of particles deposited in these regions. Churg and colleagues (*30*) have shown that particles tend to be deposited and retained at airway bifurcations, and that these are also the sites in which cancers are more likely to develop. The centriacinar zone in the human is not generally recognized as being a primary site for the genesis of lung cancers, although in recent decades there has been a marked increase in peripheral adenocarcinomas (*31*), suggesting that this region may need to be reevaluated as a potential site for the origin of lung cancers. This is also the region where lung cancers develop in rodents exposed to a variety of nonfibrous, nongenotoxic particulates (*32*).

In summary, we have shown that a combination of microdissection, histology, and semiquantitative evaluation of tissue changes is a valid approach for evaluating the effects of airborne particles on the human lung. Furthermore, we show that the principal sites of deposition of ambient particles and associated tissue remodeling are the terminal bronchioles and first-generation respiratory bronchioles. This transitional zone has unique anatomical and physiological features. The significance of lesions at this site is not currently known: however, extrapolation from studies of occupational groups and cigarette smokers would suggest that there may be long-term health effects.

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